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Impact of Cadmium on Carbohydrate Metabolism in Catfish *Mystus vittatus* (Bloch.)

T. Nalini[1] and M.V. Radhakrishnan[2]
1 Department of Zoology, Vinayaka Missions University, Salem, Tamilnadu, INDIA
2 Department of Zoology, Annamalai University, Annamalainagar, Tamilnadu, INDIA.
mvrkrishnan44@yahoo.com

ABSTRACT:
The present investigation has been conducted to study the sublethal toxic effects of cadmium (15ppm; 10% of 96LC_{50}) on carbohydrate metabolism of the freshwater fish *Mystus vittatus*. Biochemical analyses (Glucose, Glycogen and Lactic acid) was conducted after exposure of the fish for 5, 10, 20, 30, 40, 50 and 60 days intervals. The glycogen content in liver and muscle tissues showed a significant decrease. The decrease in liver glycogen and subsequent liberated glucose moieties brought about by glycogenolysis renders available metabolic to meet the energy demands of the fish subjected to toxic stress. The depletion of muscle glycogen is compensated by a mobilization of glucose from liver, translocated through blood, as the blood shows a steep hike in blood glucose.The increase in lactic acid content in muscle is transported to liver through blood for its utilization and for the resynthesis of glucose through lactic acid or Cori cycle. From all these, the carbohydrates appear as biological indicators heralding functional adaption to meet the energy demands in *M. vittatus* subjected to sublethal cadmium stress.

Key words: Cadmium toxicity, carbohydrate metabolism, *Mystus vittatus.*

INTRODUCTION

Metal pollutants are currently considered to be one of the most toxic contaminants present worldwide [1,2] as a result of human activities acting upon the natural environment with different chemicals. The aquatic environment with its water quality is considered as the main factor controlling the state of health and diseases in both man and animal. The increasing use of the waste chemical and agricultural drainage systems represents the most dangerous chemical pollutants affecting their survival, growth and reproduction. Moreover these organisms accumulate pollutants into their organ systems and have been argued that these organisms can be used as monitors of variation in pollutant concentrations [3, 4]. Heavy metals which of courses cannot be metabolized, persists in the body and extend their toxic effects by combining with one or more reactive groups essential for normal physiological functions. Since the outbreak of endemic Itai-Itai disease, Cadmium has been recognized as a major aquatic pollutant. In enters into the water bodies from combustion of fuels, and plastics, and phosphatic fertilizers, pesticides, domestic wastes, oil refineries and electroplating industries [5]. At high levels, cadmium causes kidney problems, anaemia, and bone marrow disorders. The major portion of cadmium ingested into our body is trapped in the kidneys and eliminated. A small fraction is bound most effectively by the body proteins, metallothionein, present in the kidneys, while the rest is stored in the body are gradually accumulates with age. When excessive amounts of Cd^{2+} are ingested it replaces Zn^{2+} at key enzymatic sites, causing metabolic disorders [6]. The available information on altered carbohydrate metabolism in fishes in response to toxicity studies is meager and especially to heavy metal poisons [7-15]. Hence in the present study, the glycogen, glucose and lactic acid contents in blood, liver and muscle were estimated to understand the carbohydrate metabolism and its role in the fish *Mystus vittatus* subjected to treatment with cadmium separately for 5, 10, 20, 30, 40, 50 and 60 days for sublethal concentrations.

MATERIALS AND METHODS

Irrespective of the sex healthy specimens of *M. vittatus* of 32-34g of body weight and 10-12cm length belonging to a single population were collected locally and were confined to large plastic aquaria bearing tap water for 30 days in the laboratory for acclimation. They were fed with boiled egg white on everyday (d) for 3 hours (h) before the renewal of the medium. Water was renewed after every 24 hours with routine cleaning of the aquaria leaving no faecal matter, dead fish (if any) or unconsumed food.

Prior to the commencement of experiment, 96 hours median lethal concentration (96hr LC_{50}) of cadmium chloride (99% pure, E-merck, India) was estimated following trimmed spearman Karber method [16] and 24 hr renewal bioassay

system and was found to be 150ppm after 5% trimming. For the analysis of sublethal toxicity seven groups of ten fish each were exposed separately to cadmium chloride (15ppm; 10% of 96hr LC_{50}) solution prepared in tap water. The experimental medium was prepared in tap water. The experimental medium was prepared by dissolving cadmium chloride (15mg/L) in tap water having dissolved oxygen 6.5ppm, pH, 7.4, water hardness 360mg/L and water temperature 27±2˚C [17]. Each group was exposed to 50litre of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaria containing 50 litre tape water without the addition of cadmium chloride as controls. Feeding was allowed in the experimental as well as control groups' everyday for a period of 3 hr before the renewal of the media throughout the tenure of the experiment.

After the expiry of 5, 10, 20, 30, 40, 50 and 60 days of exposure five fish each from the respective experimental as well as control groups were sacrificed. The blood samples were collected from the caudal vein with the help of 24 guage needle and stored in heparinised glass tube. The liver and muscle were excised, processed and proceeded for biochemical estimation of glucose, of blood, liver and muscle, glycogen of liver and muscle and lactic acid of blood, liver and muscle. Blood glucose was determined by the method of Murrell and Nace [18]. The blood sample (0.1ml) was collected by caudal puncture using heparinised hypodermic syringe and was immediately deproteinised in 10ml of 10% fungstic acid. The solution was filtered and the filterate was used for glucose estimation. One ml of the filterate was taken into a clean test tube and to it added 1.0ml of potassium ferricyanide (0.05%). The tubes were places in a boiling water bath for 15 seconds and cooled in running tap water. When the contents were sufficiently cooled, 1.0ml of cyanide carbonate (buffer) was added and the tubes were again placed in a boiling water bath for 15 minutes and then quickly cooled to 25-30˚C. Subsequently 2ml of ferric dupanol reagent was added to each tube, followed by the addition of 6ml of distilled water. The solution was mixed by lateral shaking and after 10 minutes, the intensity of blue colour formed was read at 640nm against a reagent black in a spectromic 20 (Bausch and Lomb). A standard solution of glucose. The unknown glucose values of blood samples were directly read from the curve. The glucose values are expressed as mg/ml of blood.

The colorimetric micro method of Kemp and Kits Van Haijigen [19] was employed for the quantitative estimation of glucose and glycogen in tissues. A known quantity of tissue was isolated and homogenized in 5ml of 80 percent methanol. The suspensions was centrifuged and the supernatant containing free glucose was decanted into a calibrated glass tube. The residue was set apart for the quantitative estimation of glycogen. To the decanted solution, approximately 10mg of activated powdered charcoal was added. The methanol was allowed to evaporate by warming the solution over a water bath. Deproteinizing solution (5g of TCA and 100mg of silver sulphate dissolved in distilled water and made upto 100ml) was added to the residual aqueous solution to bring the total volume to 5ml. the suspension was centrifuged and the clear supernatant was used for the estimation of glucose. For the estimation of glycogen, the residue left after methanol extraction was homogenized in 5ml with deprotenizing solution and later centrifuged. The clear supernatant was collected for the estimation of glycogen. One ml of the respective samples were taken in a separate test tubes and 3ml of concentrated sulphuric acid (98 percent) was added to it. The mixture was heated in a boiling water both for 6.5 minutes and subsequently cooled in running tap water. The intensity of colour developed was measured in a spectrophometer against a reagent blank at 520nm. The quantities of glucose and glycogen present in the respective samples were read the standard curve drawn previously for known quantities of the sample and the values were expressed as mg/g wet wt. of the tissues.

Blood lactic acid was determined by the method of Barker and Summerson [20]. The blood sample (0.2ml) was collected by caudal puncture using heparinised hypodermal syringe. The blood sample was immediately deproteinised with 10ml of 10% tungstic acid and the solution was filtered using Whatman No. 1 filter paper. One ml of filterate was taken in a graduated 10ml centrifuge tube and 1.0ml of 20% copper sulphate solution was added, then made upto the mark with distilled water. One gram of powdered

calcium hydroxide was added and the tube was shaken vigorously until the contents were dispersed uniformly. The tubes were kept for an hour with intermittent shaking and centrifuged. One ml of the clear supernatant was transferred to a clean, dry test tube having an internal diameter of about 18 to 23 mm and 0.05ml of 4% copper sulphate followed by 6.0ml of cold sulphuric acid were added. The contents were mixed by lateral shaking, and the tubes were kept in a boiling water bath for exactly 6.5 minutes and the cooled. 0.1 ml of p-hydroxy diphenyl was added directly to the solution, and precipitate was dispersed quickly by lateral shaking. Then the tubes were kept at room temperature for 30 minutes, they were placed in boiling water bath for 90 seconds and cooled. The colour was read at 560nm against a reagent blank in a spectrophotometer and the values were expressed as mg lactic acid/ml of blood.

Lactic acid in the tissue was determined by the method of Barker and Summerson [20]. The tissue were isolated in a cold room at 10˚C and chilled in deep freeze. After 2 to 3 hours of cooling the tissues were quickly weighed in a cold room, immediately homogenized in cold 10% TCA (1ml contain 50mg of liver or 150mg of muscle) and centrifuged at 3000rpm for 15 minutes one ml of supernatant was used for the estimation of lactic acid. The values were expressed as mg lactic acid of wet wt. of the tissue.

The data obtained for the parameters were subjected to standard statistical analysis based on random sampling of five different samples from every fish of each sampling time and their respective control groups. Duncan's multiple range test [21] was performed to determine whether the parameters altered significantly by exposure periods. Since there was no significant variations between the measurements taken from various control groups at different exposure periods the average value of all the control group was taken into account.

RESULTS AND DISCUSSION

After exposure to sublethal concentration of cadmium chloride (15ppm), the exposed specimens showed various changes in their carbohydrate contents such as glycogen, glucose and lactic acid of blood, liver and muscle are given in Figures 1-8. When *M. vittatus* was exposed to sub lethal cadmium the liver and muscle showed a decrease in glycogen content. In these tissues, it provides one of the sensitive biochemical indicators reflecting changes in the metabolic activity heralding functional adaption [22,23]. Stressful situations in fish elicit neuroendocrine responses, which in turn induces disturbances in carbohydrate metabolism [24]. The stress of acute hypoxia and the physical disturbances accompanied with rapid depletion of liver and muscle glycogen, is reflected in the experimental fishes when exposed to pollutants [25]. Fish exposed to sublethal concentration of cadmium showed a well marked and gradual decrease in liver glycogen content at all periods of exposure. This change in liver glycogen indicates that during the exposure periods of stress the demand for the extensive utilization of the energy is met by glycogen mediated through glycogenolysis. The liberated glucose moieties mobilized from liver glycogen, were transported to muscle and other organs through blood to meet the energy requirements necessitated by the accelerated movements of fish under stressful situations to adapt themselves in the toxic medium. This is reflected in the consequently steep like in the blood and liver glucose level. As with liver glycogen the muscle glycogen also showed a gradual decrease in the content, without fluctuations at all exposure periods when *M. vittatus* was treated with sublethal concentrations of cadmium. This is due to the utilization of reserve muscle glycogen to a certain extent, apart from the readily available glucose in the muscle and glucose mobilized from liver and translocated to the muscle to meet the energy requirements of the enhanced muscular activity of the fish. The increased muscular activity by the abnormal behavioural changes associated with the erratic swimming and jerky movements in the exposed toxic swimming and jerky movements in the exposed toxic medium. Moreover, the enhanced activity of succinate dehydrogenase, in the process of metabolic adjustments, is adequate enough to cope with the increase in the energy demand during the exposure periods of fish to sublethal concentrations of cadmium.

It has been reported that glucose level in blood represents a dynamic balance between the rate at which glucose enters the blood from the liver

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Fig. 1. Showing the amount of glycogen in the liver of *M. vittatus* exposed to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days

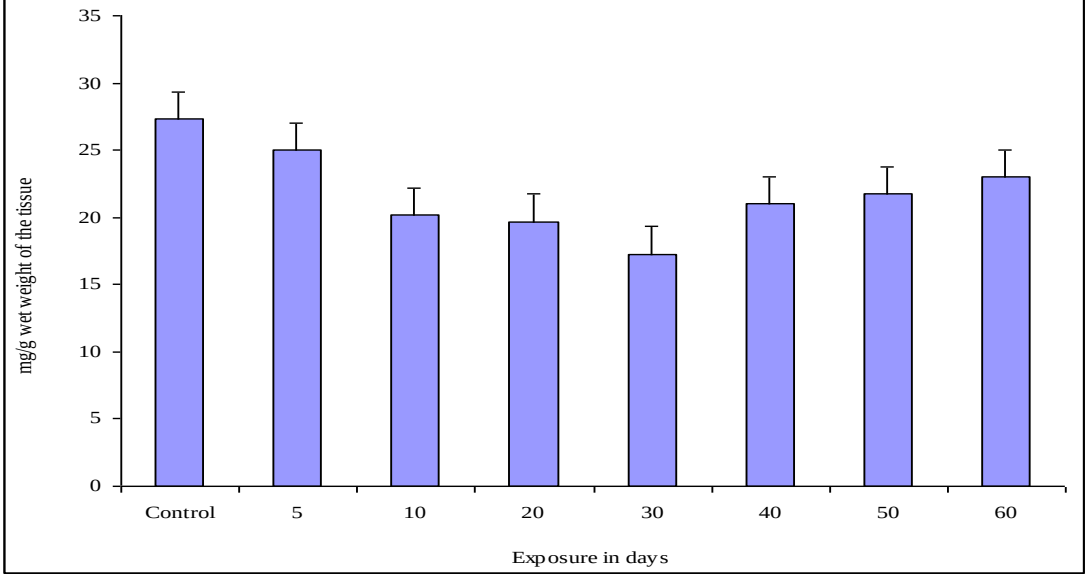
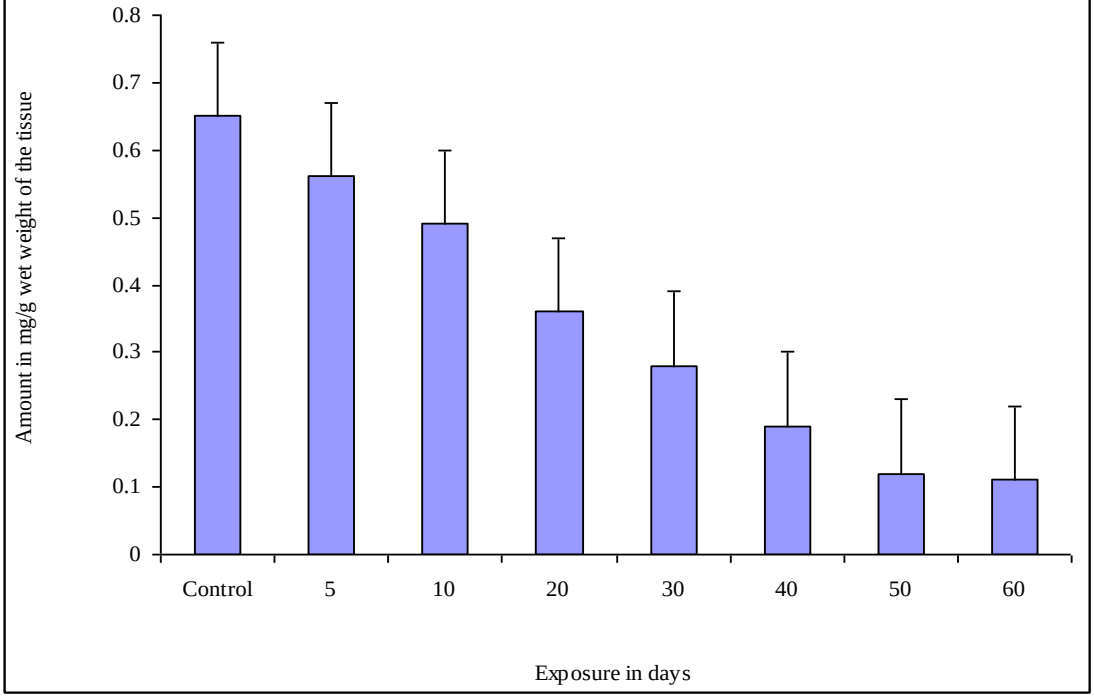


Fig. 2. Showing the amount of glycogen in the muscle of *M. vittatus* after exposure to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days



ISSN: 0975 – 8704
IJBLST (2011), 3(1):1-10

Fig.3. Showing the amount of glucose in the blood of *M. vittatus* exposed to 15 ppm of cadmium chloride for 5,10,20,30,40,50 and 60 days

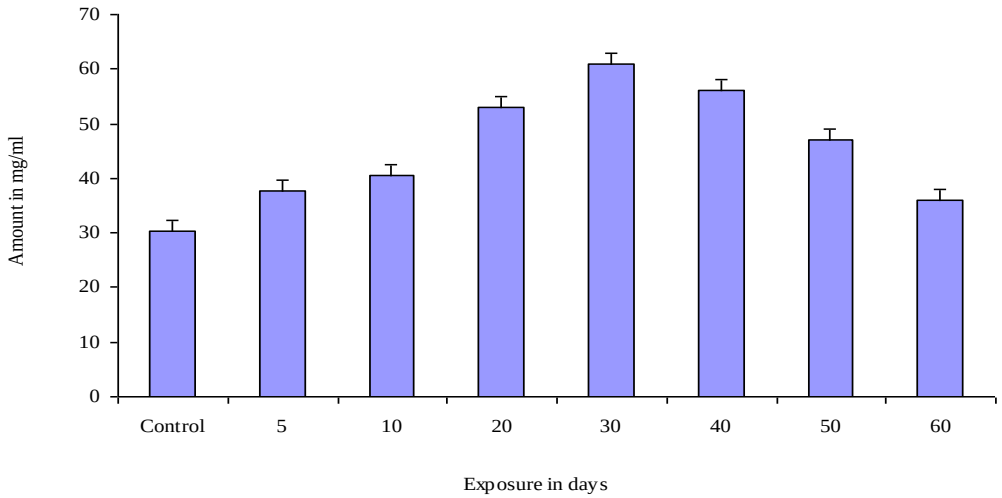
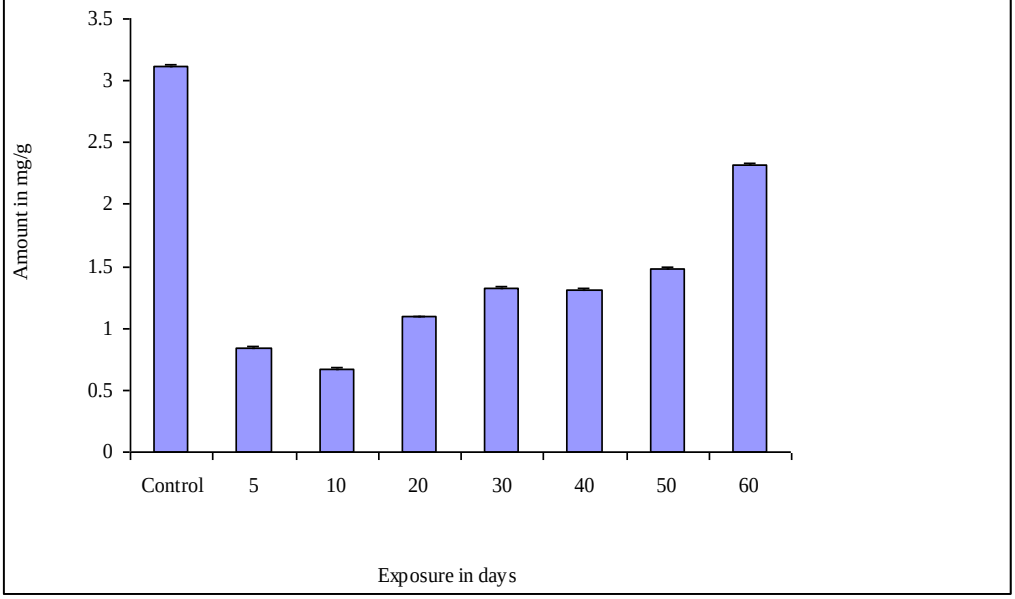


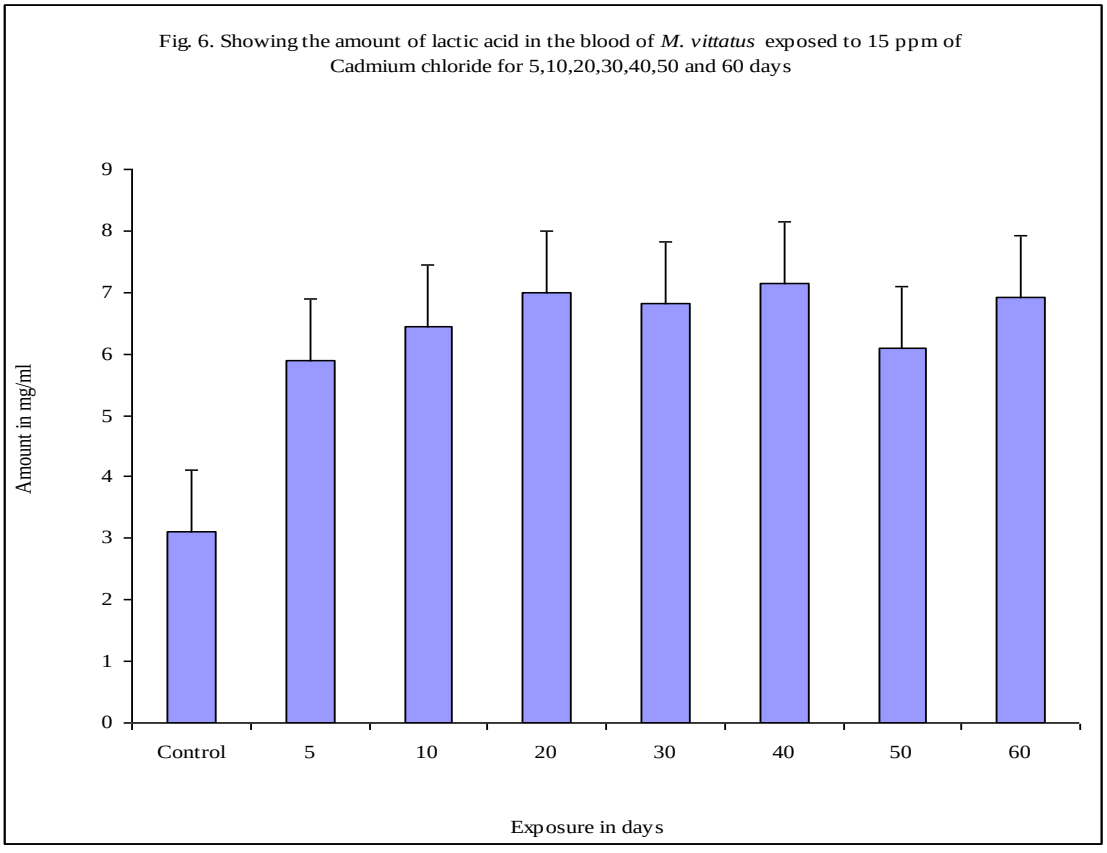
Fig.4. Showing the amount of glucose in the liver of *M. vittatus* exposed to 15 ppm of cadmium chloride treatment for 5,10,20,30,40,50 and 60 days



ISSN: 0975 – 8704
IJBLST (2011), 3(1):1-10

Fig. 5. Showing the amount of glucose in the muscle of *M. vittatus* exposed to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days

Fig. 6. Showing the amount of lactic acid in the blood of *M. vittatus* exposed to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days



ISSN: 0975 – 8704
IJBLST (2011), 3(1):1-10

Fig.7. Showing the amount of lactic acid in the liver of *M. vittatus* exposed to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days

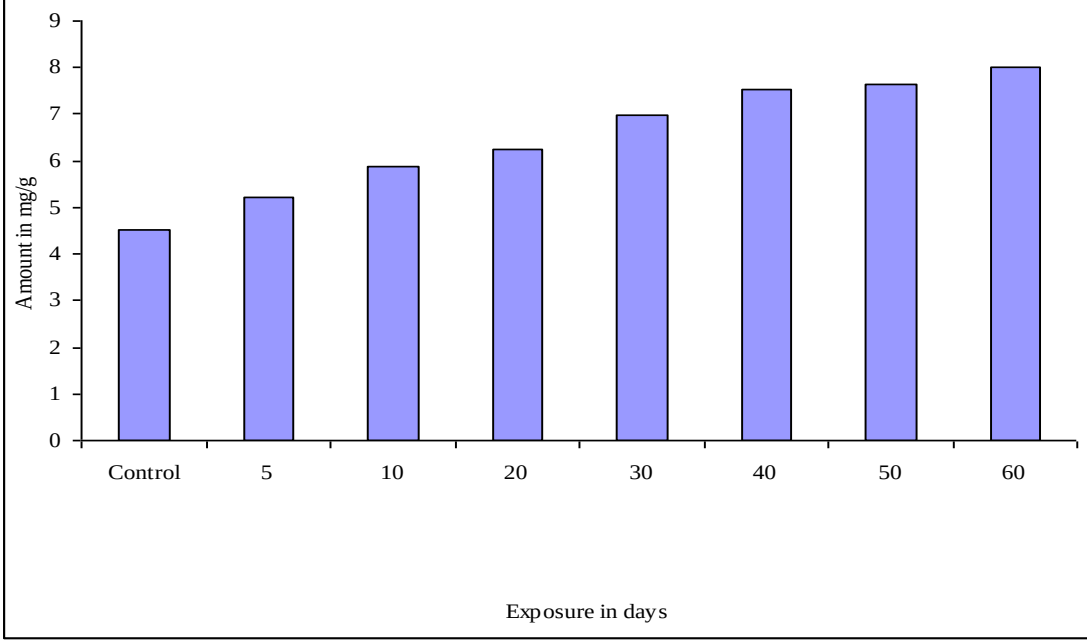
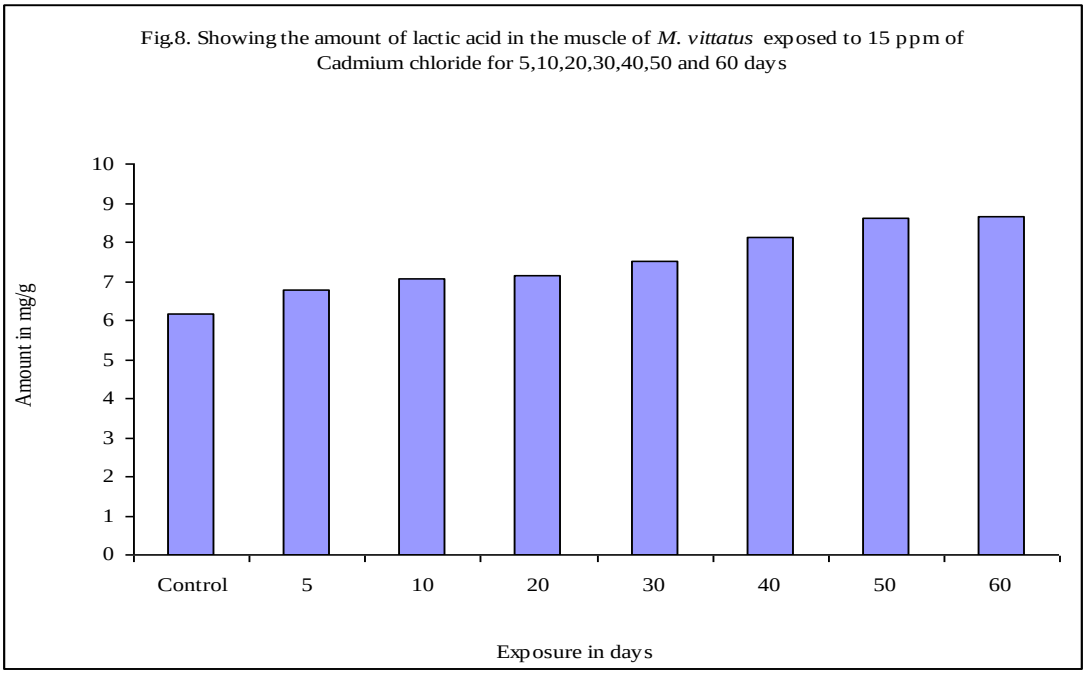


Fig.8. Showing the amount of lactic acid in the muscle of *M. vittatus* exposed to 15 ppm of Cadmium chloride for 5,10,20,30,40,50 and 60 days



and the rate at which it is being removed by the body tissues from the blood [26]. Blood glucose has been shown to be a sensitive biochemical indicator of environmental stress induced by handling, forced activity, thermal shock and contact with pollutants [27-29]. The remarkable increase in blood glucose level in *M. vittatus* exposed to sublethal concentration for different days indicate that glycogenolysis takes place in the liver whereby, the reserve glycogen is being converted into glucose which is transported to muscle and other organs through blood to meet the energy demands of the fish in altered situations. It is logical to presume that the blood

glucose, derived from liver is relatively more in fishes exposed to higher concentration of cadmium, chiefly due to the greater activity of the fish, as evidenced by the abnormal behavioural changes.

Mystus vittatus treated with sublethal concentration of cadmium produced perceptible changes in the glucose level of liver and muscle without exception, the liver glucose decreased at all periods of exposure. Mobilization of glucose from liver glucose. The decrease observed in the muscle glucose content at all periods may be due to the complete utilization of available muscle glucose and glycogen and then incoming liver glucose through blood. The liver and muscle glucose decreased as in sublethal treated fish, but the extent of decrease in more pronounced in median lethal concentration. A steep downward trend in the liver glucose content may be due to the rapid supply and greater utilization of liver glucose. An extensive utilization of glucose in the muscle may be responsible for the greater reduction of glucose at higher concentration.

Lactic acid is formed through glycolysis under anaerobic condition of glucose catabolism. In the present study, *M. vittatus* showed a well pronounced increase in the lactic acid content in blood, liver and muscle during exposure to sublethal concentration irrespective of the time of exposure. It is likely that the lactic acid, formed in the muscle and other tissues during glycolysis, might have been transported to liver via blood accounting for the hyperlactimia of blood and increased liver lactic acid level at all periods in sublethally treated fish. Because of the absence of the enzyme, glucose-6-phosphatase in the muscle and other tissues, which is necessary for the conversion of lactic acid into glucose, the lactic acid produced in the tissues is transported to the liver through blood [30]. Since liver the metabolic site, the lactic acid transported from the tissue to liver is utilized for the resynthesis of glucose and glycogen through lactic acid or Cori cycle [31] contributing to the increase in the level of lactic acid in liver and blood at all periods studied. In the toxic medium, fishes exhibited an increased muscular activity due to abnormal behavioral changes. The oxygen supplied to the muscle during these increased muscular activity is not sufficient enough to meet the oxidative requirements to fulfill the tremendous energy requirements. Therefore, glucose in the muscle tissue, under anaerobic conditions, is converted to lactic acid which in turn increases the muscle lactic acid at all stages of exposure. This may be due to the increased formation and transportation of lactic acid from the tissues to liver. The increased lactic acid accumulation in liver may be due to the increased influx of the lactic acid at 50 and 60 days when compared to earlier exposures. This suggests that during these periods the incoming lactic acid from the muscle to the liver is high or due to the accumulation of lactic acid. In short, the increase in the muscle lactic acid may be due to the severe stress imposed on the fish by the continuous exposure results in an increased lactic acid formation.

REFERENCES

[1] Athikesavan, S., Vincent, S., T. Ambrose and Velmurugan, B., (2006). Nickel induced histopathological changes in different tissues of freshwater fish *Hypophthalmiehthys molitrix* J. Environ. Biol. 27(2): 401-403.

[2] Sathyaparameswar, K., Ravinder Reddy, T and Viyayakumar N (2006). Effect of chromium on protein metabolism of freshwater mussel. *Lamellidens marginalis* J. Environ. Biol. 27(2): 401-403.

[3] Strong, M and Luoma, S. (1981). Variations in the correlation of body size with concentrations of Cu and Ag in the bivalve *Maleoma balthiea*. Can. J. S. Fish. Aquat. Sci. 38: 1054-1064.

[4] Luten, J., Bouget, W., Burggraf, M and Rus, J (1986). Accumulation, elimination and speciation of Cd and Zn in mussel *Mytilus edulis* in the natural environment Bull. Environ. Contam. Toxicol. 30: 579-586.

[5] Moore J.W., S. Ramamoorthy (1984) Heavy metals in natural waters, applied monitoring and impact assessment, Springer Verlag, New York, Berlin Heidelberg, Tokyo, p. 268

[6] De, A.K. (1996). Environmental chemistry, New Age International (P) Ltd. New Delhi. P. 364..

[7] Hodson, P.V. (1976). Temperature effects on lactate glycogen metabolism in zinc intoxicated rainbow trout *Salmo gairdneri* J. Fish. Res. Bd. Can., 82: 139.

[8] Rao, K.S.P. and K.V.R. Rao (1979). Effect of sublethal concentration of methyl parathion on selected oxidative enzymes and organic constituents on the tissues of the freshwater fish *Tilapia mossambica* (Peters). Curr. Sci., 48: 526.

[9] Gill, T.S. and J.C. Pant (1981). Effects of sublethal lethal concentrations of mercury on a teleost *Puntius conchonius*: Biochemical and haematological responses. Ind. J. Exp. Biol. 19: 571-573.

[10] Bakthavathsalam, R. and Y. Srinivasan Reddy, (1982). Changes in the content of glycogen and its metabolities during acute exposure of *Anabas testudineus* (Bloch) to furadan. J. Biosci. 4, 19-24.

[11] Garania, S.N. (1984). The effect of heavy metals on certain elements of carbohydrate metabolism in *Caspian gammaridae*. Gidrobiol. Zn., 20: 67-69.

[12] Torres, P., L. Tort, J. Planas and R. Fios (1986). Effect of confinement and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula*. Comp. Biochem. Physiol., 83C (1): 89-92.

[13] Jha, B.S. and S. Pandey (1989). Alternation in the total carbohydrate level of intestine, liver and gonad induced by lead nitrate in the fish *Channa punctatus*. In: Environmental risk assessment (Eds: Y.N. Sahat, P.N. Deshmukh, of Environmental Biology, pp. 207-211.

[14] Ober, A.G., Gonalez, M and Santa Maria, (1987). Heavy metals in molluscan, gustacean and other commercially important Chilean marine coastal water species. Bull. Environ. Contam. Toxicol. 38: 534-539.

[15] Muley, D.V. Karanjkar, D.M. and Maske S.V. (2007). Impact of industrial effluents on the biochemical composition of freshwater fish *Labeo rohita* J. Environ. Biol. 5, 28(2): 245-249.

[16] Hamilton, M.A., Russo, R.C. and Thurston, R.V. (1977). Trimmed spearmen Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714-719.

[17] APHA (1998) Standard method for the examination of water and wastewater. 20th edn. *American Public Health Association*, New York, USA.

[18] Murrell, L.R. and P.F. Nace (1958). Determination of glucose in fish blood. A modification of the folin malmros micro procedure. Lan. J. Biochem. Physicol. 36(11): 1121-1124.

[19] Kemp, A. and J.M. Kits Van Heijhigeri, (1954). A chlorimetric method for the determination of glycogen in tissue. Biochem. J., 56; 646-648.

[20] Barker, S.B. and W.H. Summerson, (1941). The chloriometric determination of lactic acid in biological material. J. Biol. Chem., 138. 535-554.

[21] Brunning, J.L. and Kintz, B.L. (1968). Computational handbook of statistics Fores men and company, Dallas, U.S.A. p. 308.

[22] Mustafa, S. and A.K. Jafri, (1981). Fat and glycogen concentration in flesh of catfish *Heteropneustes fossilis* (Bloch) as indicators of living condition. Curr. Sci., 50(18).

[23] Metelev, V.V., A.L. Kanaev and N.G. Dzasokhova, (1983). Water toxicology. Amerinol Publishing Co. Pvt. Ltd., New Delhi. Pp. 56-60.

[24] Mazeaud, M.M., F. Mazeaud and C.M. Donaldson, (1977). Primary and secondary effects of stress in fish. Some new data with general review. Trans. Amer. Fish. Soc., 106-201.

[25] Murthy, A.S. and A. Priyamada Devi, (1982). The effects of endosulfan and its isomers on tissue of endosulfan and its isomers on tissue protein, glycogen and lipids in the fish *Channa punctatus*. P.B.P. Vol 17(3): 280-283.

[26] Saskin, S. (1941). The blood sugar: Its origin regulation and utilization. Physiol. Rev. 21: 140-201.

[27] Nakano, T and N. Tamtinson, (1967). Catecholamines and carbohydrate concentrations in rainbow trout *Salmo gairdneri* in relation to physical disturbance. J. Fish. Res. Biol. Can. 24: 1701-1715.

[28] Hill, C.W. and P.O. Fromm, (1968). Response of the adrenal gland of rainbow trout *Salmo gairdneri* to stress. Gen. Comp. Endocrinol., 11: 69-77.

[29] Wedmeyer, G.A. and W.T. Yasutake, (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Fish and wildlife service Technical paper 89, U.S.D.I. Washington.

[30] Ambika Shanmugam (1980). Fundamentals of biochemistry for medical students, Navabharat Offset works, pp. 350-411.

[31] Mayer Bodensky, (1947). Introduction to physical chemistry. Publ. John Wiley and Sons, New York, pp. 314-462.